



EL

Electrace: een plug- and-play biosensor

iGEM TU Delft-Leiden




Wat is iGEM?

- International **G**enetically **E**ngineered **M**achine competition
- 245 teams
- 10de editie
- Jamboree
- Wiki
- Policy & Practice
- Biobricks



Registry

 tools catalog repository assembly protocols learn login 0

Registry of Standard Biological Parts

main page design experience information part tools **edit**

Part:BBa_K1316000

Reporter

Not Released

Sample Not in stock

No Results

-1 Uses

Get This Part

Designed by: Joan Cortada Garcia Group: iGEM14_TU_Delft-Leiden (2014-08-24)

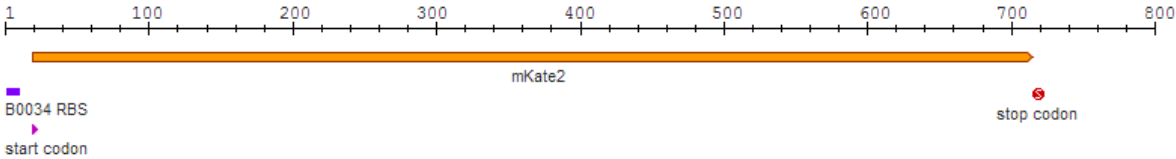
mKate2 protein

mKate2 is a far-red fluorescent protein used with reporting purposes. mKate2 codon usage is optimised for high expression in mammalian cells (humanised). It is, nevertheless, suitable for propagation in *E. coli*.

Sequence and Features

Subparts | **Ruler** | [SS](#) | [DS](#)

Length: 717 bp [View plasmid](#) [Get part sequence.](#)



Assembly Compatibility: 10 12 21 23 25 1000

Parameters

Categories

[\[edit\]](#)

Characterization

igem tools catalog repository assembly protocols learn BBa_ login 0

Registry of Standard Biological Parts

For more info, visit [TU Delft iGEM13 Wiki](#)

Cleavage of SUMO from Peptide by Ulp-1 protease

Introduction:

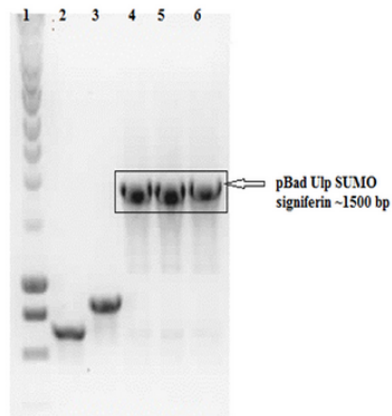
The SUMO-peptide helps in increasing the soluble fraction of peptide but peptides are not biologically active in a fusion, they have to be cleaved from the fusion to get an active peptide fraction. This was achieved by in vivo production on SUMO specific Ulp-1 proteases.

The biobrick BBa_K1022117 was constructed in such a way that the SUMO-peptide production was driven by the strong T7 phage promoter and the Ulp-1 production was driven by arabinose inducible promoter pBad. The plasmid was transformed into an BL21(DE3) pLysS strain. This construct was designed to check whether in vivo cleavage is possible. The main idea of the experiment is to first produce large amount of soluble fraction of SUMO-peptides and then produce the Ulp protease to cleave the sufficiently produced fusion peptides.

The protocol can be seen [here](#).

Result:

The presence of plasmid with gene inserts encoding the SUMO-peptide (BBa_K1022116/117/118) was confirmed by a colony PCR. The expected size of the insert was approximately around ~1500 bp which was clearly evident from the agarose gel picture figure below. Though the actual size of the inserts are ~1300 bp the use of sequencing primers VF2 and VR which bind 100-150 base pair away from suffix and prefix the bands are around 1500 bp. An Eurogentec Smartladder MW-1700-10 [lane 1] (<https://secure.eurogentec.com/uploads/TDS-MW-1700-10.pdf>) was used to identify the size of the fragments.



pBad Ulp SUMO peptide Colony PCR

Discussion:

The cleavage of the peptide from the SUMO is more crucial to free the peptide and make it biologically active. But if we look into the structure of the peptides, they are quite hydrophobic to be present as free peptides in the medium. So, a classical SDS page analysis is not suitable for these peptides. This made us to analyse our whole cell lysates with tandem MS approach. The cell lysates devoid of debris were subjected to MS/MS measurements, which gave a 40 % sequence coverage to the SUMO without the peptide as in figure below. The free peptide was not present intact in the solution. This could be attributed to the hydrophobicity of the free

SynBio

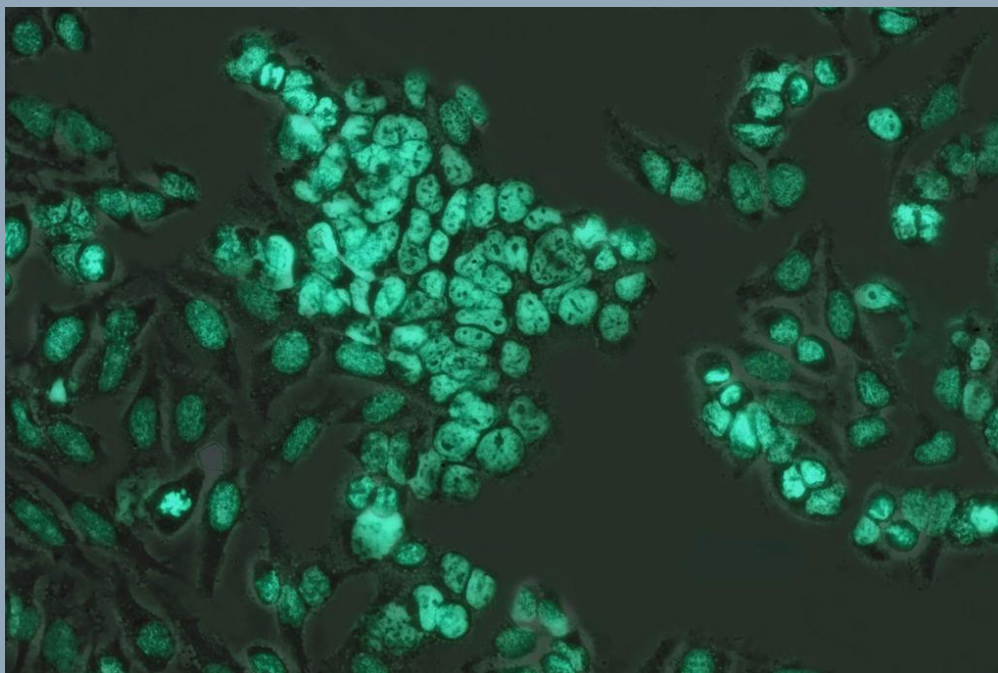
- Veel academische ontwikkeling
- SynBio: geen consumentenproducten
- Onbekend maakt onbemind

Biosensors

- Selectief
- Gevoelig
- Meer dan 800 biosensors binnen iGEM
- Toepassing blijft uit

Biosensors

- Conventionele biosensor output
 - Luminescentie + Fluorescentie
 - Laboratoriumonderzoek



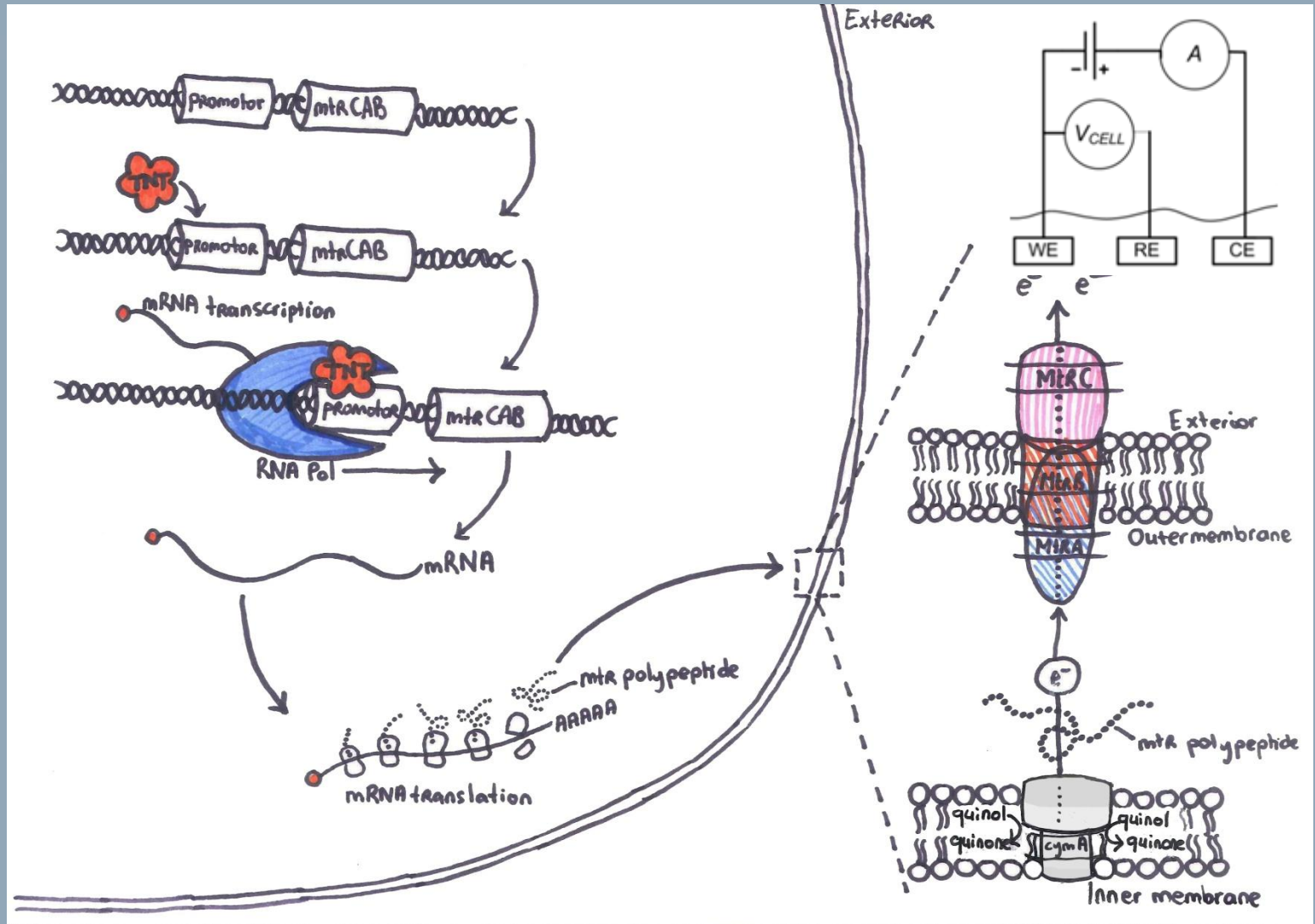
**EL**

Electrace

- Doel: genereren elektrisch signaal als respons op meting
- Plug-and-Play

EL

Electrace



EL

Electrace

- Electrace output
 - Potentiostat
 - Elektrische stroom
 - Buiten het lab
 - Handheld device



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Electrace

Plug and Play biosensor system



Software developers
make add-ons for
each new biosensor
strips a web app

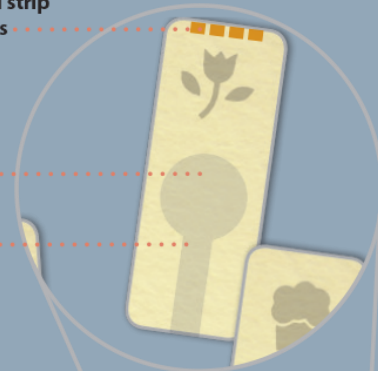


Wetware developers
create new biosensor
strips (by plugging
in biobricks to the
Electrace "chassis")

Laminated carboard strip
with copper contacts

Immobilised
Electrace E coli

Paper
Microfluidics



Wifi device reads Electrace
current and sends data to
web app over wifi.



Landmijnen detecteren

- Proof of Principle
- Promoter gevoelig voor TNT in grondwater
- Goedkoop, handzaam alternatief/aanvulling op huidige opsporingsmethoden



Discussie

- Product: Biosensor op een chip
- Hoe komen wij van een werkend prototype naar een commerciële product?

Discussie

- GGO buiten lab
 - Toepassing door leken vs. Veiligheid
- Robuust vs. evolutionaire competitie
- Open source development vs. IP
 - €€€
 - Aansprakelijkheid